

Patent claims:

1. DNA molecule which comprises the genes for biosynthesizing acarbose.
2. DNA molecule according to Claim 1, which comprises the genes for biosynthesizing acarbose and homologous pseudo-oligosaccharides.
3. DNA molecule according to either Claim 1 or 2, characterized in that the genes are arranged, with respect to their direction of transcription and order, as depicted in Figure 3.
4. DNA molecule according to one of Claims 1, 2 or 3, characterized in that it exhibits a restriction enzyme cleavage site pattern as depicted in Figure 3.
5. DNA molecule according to one or more of Claims 1 to 4, characterized in that
 - (a) it comprises a DNA sequence according to Table 4, or parts thereof; or
 - (b) it comprises a DNA sequence which is able to hybridize, under stringent conditions, with the DNA molecule according to (a), or parts thereof; or
 - (c) it comprises a DNA sequence which, because of the degeneracy of the genetic code, differs from the DNA molecules according to (a) and (b) but which permits the expression of the proteins which can be correspondingly expressed using the DNA molecules according to (a) and (b), or parts thereof.
6. DNA molecule according to Claim 5, characterized in that it comprises, as the sequence mentioned under (a), the DNA sequence of nucleotides 1 to 720 (acbA gene) according to Table 4, or parts thereof.
7. DNA molecule according to Claim 5, characterized in that it comprises, as the sequence mentioned under (a), the DNA

sequence of nucleotides 720 to 2006 (acbB gene) according to Table 4, or parts thereof.

- 5 8. Recombinant DNA molecule according to Claim 5, characterized in that it comprises, as the sequence mentioned under (a), the DNA sequence of nucleotides 2268 to 3332 (acbC gene) according to Table 4, or parts thereof.
- 10 9. Recombinant DNA molecule according to Claim 5, characterized in that it comprises, as the sequence mentioned under (a), the DNA sequence of nucleotides 3332 to 4306 (acbD gene) according to Table 4, or parts thereof.
- 15 10. Recombinant DNA molecule according to Claim 5, characterized in that it comprises, as the sequence mentioned under (a), the DNA sequence of nucleotides 4380 to 5414 (acbE gene) according to Table 4, or parts thereof.
- 20 11. Recombinant DNA molecule according to Claim 5, characterized in that it comprises, as the sequence mentioned under (a), the DNA sequence of nucleotides 5676 to 6854 (acbF gene) according to Table 4, or parts thereof.
- 25 12. Oligonucleotide primer for the PCR amplification of the DNA molecule according to Claim 5.
13. Oligonucleotide primer according to Claim 12 which has the sequence according to Table 1.
- 30 14. Vector, which comprises a DNA molecule according to one or more of Claims 1 to 11.
15. Vector according to Claim 14 for use in a process for eliminating or altering natural acarbose biosynthesis genes in an acarbose-producing microorganism.
- 35 16. Vector according to Claim 15, characterized in that it is selected from the group consisting of pGM160 or related vectors.

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17. Vector according to Claim 14, characterized in that it is an expression vector and said DNA molecule is linked operatively to a promoter sequence.
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18. Vector according to Claim 17, which is suitable for expression in host organisms which are selected from the group consisting of *E. coli*, *Bacillus subtilis*, *Streptomyces*, *Actinoplanes*, *Ampullariella* and *Streptosporangium* strains, *Streptomyces hygroscopicus* var. *limoneus*, *Streptomyces glaucescens* and also *Aspergillus niger*, *Penicillium chrysogenum* and *Saccharomyces cerevisiae*.
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19. Vector according to Claim 17, which is suitable for expression in *Streptomyces glaucescens* GLA.O or *Actinoplanes* sp.
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20. Host cell which is transformed with a DNA molecule according to one or more of Claims 1 to 11 or a vector according to one of Claims 14 to 19.
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21. Host cell according to Claim 20, characterized in that it is selected from the group consisting of *E. coli*, *Bacillus subtilis*, *Streptomyces*, *Actinoplanes*, *Ampullariella* or *Streptosporangium* strains, *Streptomyces hygroscopicus* var. *limoneus* or *Streptomyces glaucescens*, and also *Aspergillus niger*, *Penicillium chrysogenum* and *Saccharomyces cerevisiae*.
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22. Host cell according to Claim 21, characterized in that it is selected from the group consisting of *Streptomyces glaucescens* GLA.O and *Actinoplanes* sp.
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23. Protein mixture which can be obtained by expressing the genes of the gene cluster according to one or more of Claims 1 to 5.
24. Isolated protein, which can be obtained by expressing a gene according to one or more of Claims 6 to 11.
25. Protein (*acbA* gene product), which is encoded by a DNA according to Claim 6.

26. Protein (acbB gene product), which is encoded by a DNA according to Claim 7.
- 5 27. Protein (acbC gene product), which is encoded by a DNA according to Claim 8.
28. Protein (acbD gene product), which is encoded by a DNA according to Claim 9.
- 10 29. Protein (acbE gene product), which is encoded by a DNA according to Claim 10.
- 15 30. Protein (acbF gene product), which is encoded by a DNA according to Claim 11.
31. Process for obtaining the proteins according to one of Claims 23 to 30, characterized in that
- 20 (a) the proteins are expressed in a suitable host cell, and
- (b) are isolated.
32. Process according to Claim 31, characterized in that the host cell is selected from the group consisting of *E. coli*, *Bacillus subtilis*, *Streptomyces*, *Actinoplanes*, *Ampullariella* or *Streptosporangium* strains, *Streptomyces hygroscopicus* var. *limoneus* or *Streptomyces glaucescens*, and also *Aspergillus niger*, *Penicillium chrysogenum* and *Saccharomyces cerevisiae*.
- 25 33. Process according to Claim 31, characterized in that the host cell is selected from the group consisting of *Streptomyces glaucescens* GLA.O and *Actinoplanes* sp.
- 30 34. Process for preparing acarbose, characterized in that
- 35 (a) one or more genes according to one or more of Claims 6 to 11 are used for expression in a suitable host cell, and
- (b) the acarbose is isolated from culture supernatants of said host cell.

35. Process for preparing acarbose according to Claim 34, characterized in that host cells according to one of Claims 21 or 22 are selected.
- 5 36. Process for preparing acarbose, characterized in that
(a) one or more genes according to one or more of Claims 6 to 11 are eliminated in a natural acarbose-producing host cell, and
(b) the acarbose is isolated from said host cell.
- 10 37. Process for preparing acarbose according to Claim 36, characterized in that host cells according to Claim 22 are selected.
- 15 38. Process for preparing acarbose, characterized in that a process according to one of Claims 34 to 35 is combined with a process according to one of Claims 36 to 37.
- 20 39. Process for completing the gene cluster for biosynthesizing acarbose according to Claim 5, characterized in that
(a) adjoining genomic DNA regions are isolated by hybridization methods using hybridization probes which are derived from the DNA molecule according to Claim 5, and
(b) are sequenced.
- 25 40. Process for completing the gene cluster for biosynthesizing acarbose according to Claim 5, characterized in that
(a) adjoining genomic DNA regions are isolated by means of PCR using PCR primers which are derived from DNA sequences from the DNA molecule according to Claim 5 and primers which possess a sequence which permits hybridization to sequences of the vector system employed, and
(b) are sequenced.
- 30 41. Process for isolating a gene cluster for biosynthesizing acarbose and homologous pseudo-oligosaccharides from acarbose-producing microorganisms other than *Streptomyces glaucescens* GLA.O,

characterized in that, proceeding from the recombinant DNA molecule according to Claim 5,

- 5 a) hybridization probes are prepared,
 b) these hybridization probes are used for the genomic or cDNA
 screening of DNA libraries which have been obtained from
 the corresponding microorganism, and
 c) the clones which are found are isolated and characterized.

10 42. Process for isolating a gene cluster for biosynthesizing acarbose
 and homologous pseudo-oligosaccharides from acarbose-producing
 microorganisms other than *Streptomyces glaucescens* GLA.O,
 characterized in that, proceeding from the recombinant DNA
 molecule according to Claim 5,

- (a) PCR primers are prepared,
15 (b) these PCR primers are used for accumulating DNA
 fragments of genomic DNA or cDNA from a corresponding
 microorganism,
 (c) the accumulated fragments are isolated and characterized,
 and
20 (d) where appropriate, are employed for a process according to
 Claim 41.

25 43. Process according to Claim 41 or 42, characterized in that the
 microorganisms are selected from the group consisting of
 Actinomycetales, *Streptomyces*, *Actinoplanes*, *Ampullariella* and
 Streptosporangium strains, *Streptomyces hygrosopicus* var.
 limoneus and *Streptomyces glaucescens*.

30 44. Process according to Claim 43, characterized in that the
 microorganisms are selected, in particular, from the group consisting
 of *Streptomyces glaucescens* GLA.O and *Actinoplanes* sp.

45. Use of *Streptomyces glaucescens* GLA.O for obtaining acarbose.

35 46. Use of *Streptomyces glaucescens* GLA.O for preparing mutants of
 this strain which permit more abundant acarbose production.

47. Process for altering the gene expression of endogenous acarbose biosynthesis genes in order to obtain an improved acarbose yield, in which
- 5 a) mutations are introduced in one or more of the respective gene promoters, and
- b) the acarbose yield of the resulting producer strain is compared with that of the starting strain.
48. Process according to Claim 47, characterized in that the mutations
- 10 are
- a) transitions
- b) deletions and/or
- c) additions.

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